

Biochemistry

PURIFICATION OF RECOMBINANT BOVINE DEOXYHYPUSINE SYNTHASE

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The mature eIF-5A is the only protein known to contain the unusual amino acid hypusine. The synthesis of hypusine is important for the biological activity of eIF-5A. Hypusine synthesis in mature eIF-5A is a unique two-step posttranslational modification involving deoxyhypusine synthase and deoxyhypusine hydroxylase. Previously, others in this lab has cloned, expressed and characterized recombinant bovine deoxyhypusine synthase (rbDS). The activity of rbDS was found much greater than that of recombinant human deoxyhypusine synthase (rhDS). We are interested in the underlying reasons for the difference. Large quantities of the rbDS are needed for structural determination and for complexation with eIF5A.

In this study, we optimized the conditions for overexpression of rbDS in BL21 DE3 host cells. The T7-tagged rbDS was purified from cell-free extract by T7 antibody affinity chromatography. The purity of the protein was checked by SDS-polyacrylamide gel electrophoresis. The protein was subsequently concentrated to 5-6 mg/ml. The structure of the protein will be determined by x-ray crystallography.

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